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Etiological study of molt abnormalities in the African penguin, Spheniscus demersus

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Etiological study of molt abnormalities in the African penguin, *Spheniscus demersus*

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ABSTRACT

An important life history event for African penguins is the annual molt, which restores healthy plumage for proper thermoregulation and waterproofing. This study examined three, not necessarily mutually exclusive, hypotheses to explain the etiology of improper molting in zoo and aquarium populations: (1) the syndrome is hereditary; (2) birds do not amass enough nutritional resources prior to the molt to allow the molt to progress normally; (3) the timing of the molt is disrupted by exogenous cues that are contrary to those experienced in the wild. A two-part survey was completed by 17 U.S. zoos and aquaria that returned information on individual birds and colony management. Abnormal molters represented 14.0% in 2012 and 13.5% in 2013 of the pooled population of African penguins held at respondent institutions. Sex, along with number of eggs laid within six months prior to molt, prevalence of sires or dams molting abnormally, or colony management practices did not have significant effects on the prevalence of abnormal molting. Normally molting birds gained significantly more weight prior to molt than did birds which molted abnormally. Additionally, birds that molted abnormally were significantly older than those which molted normally. It is thus hypothesized that as birds age, they are unable to maintain effective concentrations of hormones, in particular T_3 and T_4, which in turn affects pre-molt weight gain and therefore results in an abnormal molt. Uncovering the etiologies behind abnormal molting helps humans support both wild and zoo and aquarium based populations. It is suggested that further research be conducted on the cause(s) of low pre-molt weight gain and if hormone concentration and maintenance may be a factor in abnormal molts.

INTRODUCTION

Factors such as climate change, pollution, lack of food, and oil spills have led to the decline of most of the 18 species of penguins (family Spheniscidae) around the world (Association of Zoos and Aquariums [AZA] Penguin Taxon Advisory Group [TAG], 2014). In particular, the African penguin (*Spheniscus demersus)* population has dramatically declined by over 70% in the past 11 years (Pichegru *et al*., 2013). Since the 1960s, overfishing of pelagic fish resources, the African penguin's key dietary staple, has greatly contributed to the population decline (Shelton *et al*., 1984). In 2000, over 19,000 African penguins were affected by the *MV Treasure* oil spill and another 19,500 penguins had to be relocated to prevent them from becoming oiled (Wolfaardt *et al*., 2001). With only 26,000 breeding pairs remaining by 2009, the African penguin population is still in decline (Crawford *et al*., 2011). In 2010, the African penguin was placed on the endangered species list (BirdLife International, 2013).

An important life history event for penguins is the annual molt, which restores healthy plumage for proper thermoregulation and waterproofing. Unlike most birds that only molt a few feathers at once, penguins undergo an annual catastrophic molt in which they replace the entirety of their plumage over an average of 17 days (AZA Penguin TAG, 2005; Brasso *et al*., 2013). The failure to properly molt leaves a penguin vulnerable to hypothermia due to the lack of efficient thermoregulation. An abnormally molting penguin is also vulnerable to wasting away, due to the animal's inability to swim and obtain its primary food source (fish), coupled with increased energy demands associated with shivering in order to maintain homeothermy (Groscolas and Cherel, 1992; Mazzaro *et al*., 2013; Otsuka *et al*., 2004). An incomplete or protracted molt thus threatens a penguin's life.

On average, 1.7 ± 0.2% (mean ± SE) of African penguins rescued and rehabilitated in the Western Cape of South Africa by The South African Foundation for the Conservation of Coastal Birds (SANCCOB) molted abnormally between the years of 2001-2011 (SANCCOB, unpublished data). Birds that molt abnormally are characterized by not replacing their feathers after feather loss or by not molting at all during the expected time of molt. An average of 49.7 ± 8.1% of the birds that molted abnormally died within the year, with mortality rates as high as 100% in some years.

African penguins housed in zoos and aquaria face similar hardships when experiencing abnormal molts. Although birds are still offered food, they can experience shivering from decreased insulation, a sedentary lifestyle from the inability to swim, and isolation as their compromised state can impact interaction with other penguins (Schaller, 2009). With the wild penguin population in quick decline, ensuring the health and robustness of the African penguin population in zoos and aquaria is a strategy for survival of the species. Maintaining a genetically diverse population of African penguins in zoos and aquaria provides the opportunity to conduct observational, behavioral, physiological, or genetically based research on the species, which can then be used to support wild population conservation efforts (*e.g*., Saving Animals from Extinction [SAFE], AZA, 2015). For example, research conducted at the Cologne Zoo provided insight into what behaviors indicate the onset of the breeding season in the critically endangered Philippine crocodile (*Crocodylus mindorensis*, Schneider *et al*., 2014); this information could be used in the species' conservation breeding efforts. Zoo and aquarium populations also serve as ambassadors for their wildlife counterparts and can assist in educating the public about conservation concerns and needs (Fernandez and Timberlake, 2008).

Documenting the etiology of abnormal molting in the African penguin may yield actionable recommendations that can be offered to veterinary and penguin care teams regarding preventive care and treatment. Additionally, this information can guide wildlife managers to support elements in African penguins' habitats that are conducive to proper molting (*e.g*., seasonal management of prey resources). Ultimately, uncovering the etiology of abnormal molt helps humans to support both wild and zoo- and aquarium-based populations of this endangered species.

Four not necessarily mutually exclusive hypotheses are proposed to explain the etiology of improper molting of African penguins in zoos and aquaria: (1) the syndrome is hereditary; (2) birds do not amass enough nutritional resources prior to the molt to allow the molt to progress normally; (3) the timing of the molt is disrupted by exogenous cues that are contrary to those experienced in the wild; and/or (4) abnormalities in hormone secretion and maintenance interfere with the normal progression of the molt.

Genetics

Research suggests a genetic component to molt in other avian species such as African stonechats (*Saxicola torquata axillaris,* Helm and Gwinner, 1999). The Stonechat postjuvenal molt timing is determined polygenically and within subspecies selection of genetic variation may fine tune the molt timing in relation to local environments. Mystic Aquarium has tracked a pattern of heritability regarding the occurrence of abnormal molting among its African penguin population (Mystic Aquarium, unpublished data), suggesting that molting abnormalities may be linked to a recessive allele. Pedigrees along with statistical testing can be used to determine if a trait is heritable, and if so whether it follows an autosomal, sex-linked, or a more complex mode of inheritance.

Bioenergetics

Molting is an energetically expensive process which requires metabolic adaptations, including the buildup, storage, and use of fat, as well as protein catabolism (Cornelius *et al*., 2011; Groscolas and Cherel, 1992; Mazzaro *et al*., 2013). Considering that the penguin's ability to swim and obtain food will be disrupted, the penguin must have enough fat storage to provide energy throughout the molt. In fact, there is a significant decrease in body mass (45%) and protein (50%) during the molt of Emperor and King penguins (*Aptenodytes forsteri*, *Aptenodytes patagonicus,* Groscolas and Cherel, 1992). The timing of the molt relative to breeding, nesting, and chick rearing, all of which occur on an annual basis, is an important consideration in regards to the bird's energy demands. Breeding and molting periods cannot take place at the same time due to the energy demands of each event (Otsuka *et al*., 2004; Payne, 1972). There is thus a small window of time available for penguins to complete the molt when considering the pre-molt fattening period and the time it takes to breed and raise offspring (Kemper *et al*., 2008). In order to ensure a healthy molt progression, a weight gain appropriate to sustain the individual must take place prior to the molt.

Exogenous Cues

Exogenous cues such as temperature and photoperiod can provide important cues for animals to help regulate a variety of processes such as molt, hibernation, breeding, etc. For instance, initiation of breeding season and the fertility of female sheep (*Ovis* *aries*) can be impacted by the temperature at which they are kept (Dutt and Bush, 1955). A difference in temperature contrary to what is typically experienced could disrupt the initiation or maintenance of a physiological process such as the molt. For example, white-footed mice (*Peromyscus leucopus)* exposed to cold experienced a molt that was two weeks earlier than control mice. The recommended temperature ranges for African penguins living in an enclosure is from 4.5 to 26.5°C (AZA Penguin TAG, 2005). African penguins in the wild experience water temperatures that are approximately 13.4°C (Cohen and Tyson, 1995). It may be possible that African penguins housed in AZA facilities are exposed to a water temperature range that is too broad. It may be necessary for the birds to experience a water temperature closer to what is normally experienced in the wild, coupled with seasonal temperature changes to induce molt.

Evidence also suggests that the molt in other penguin species, such as the Humboldt penguin (*Spheniscus humboldti*), is influenced by photoperiod (Scholten, 1988). Unnatural photoperiods may elicit molt rhythm and pattern irregularities in the White-crowned Sparrow (*Zonotrichia leucophrys,* Chilgren, 1978). Furthermore, a study of the Chaffinch (*Fringilla coelebs*) suggests that molt cycle and/or rate can be modified through alteration of photoperiods (Dolnik and Gavrilov, 1980). While many zoos and aquariums use photoperiod schedules that are approximate to the latitude in which the species are found (AZA Penguin TAG, 2014), it is possible that photoperiod regimes may need to be revisited.

Examination of the types of exhibits in which African penguins are housed, the lighting type and schedule, temperature regime to which they are exposed, and the institutions' breeding and molting management practices can illuminate potential effects of exogenous cues on the molt process of resident African penguins.

Hormonal Regulation

Data show that hormone concentrations vary during molting and non-molting periods. The thyroid hormone thyroxine (T_4) has a significant impact on molt regulation in penguins. During the pre-molt stage, T_4 rises and stimulates new feather growth (Groscolas and Cherel, 1992; Otsuka *et al*., 2004). Testosterone and estradiol concentrations are typically lowest in males and females during the pre-molt and molt period as this is thought to allow for the production and release of T_4 (Otsuka *et al*., 2004). The release of testosterone and estradiol is thought to effect normal molt progression as it relates to the times of breeding activities (Otsuka *et al*., 2005). Also, the stress hormone corticosterone is ordinarily suppressed during the molt (Romero *et al*., 2005), as one of the downstream effects of corticosterone is the breakdown of proteins to provide energetic resources in a "fight-or-flight" scenario, contrary to the needs of protein catabolism for proper molting. Corticosterone can inhibit feather regrowth in captive European starlings (*Sturnus vulgaris*) and white-crowned sparrows (*Zonotrichia leucophrys*) through its degradation of proteins and inhibition of protein synthesis (Romero *et al*., 2005). Contrasting the levels of thyroxine, testosterone, estradiol, and corticosterone during and outside the molting period between normally and abnormally molting birds could provide insight into whether or not hormones are a factor in abnormal molting.

This study examines hypotheses 1-3 in a citizen science survey with the participation of zoos and aquaria throughout the United States.

METHODS

Survey Population, Recruitment, and Management

Two e-mail invitations were sent out to penguin-holding zoos and aquaria represented in AZA's Penguin TAG (n = 112) via a list-serv. Interested participants were asked to email a representative (V. Rivera), who replied to individual institutions by sending the survey (developed by members of Mystic Aquarium) and associated instructions, and maintained contact with respondents to ensure survey completion. V. Rivera also conducted quality inspection of submitted data and followed up with respondents to clarify responses in question.

Data Collection

A spreadsheet composed of three sections (general institutional contact information, an individual penguin survey, and a colony survey) was developed in Microsoft Excel (v. 10, Microsoft Corp., Redmond, WA) and used to gather data. Institutions completed surveys for two retrospective years, 2012 and 2013. A representative from the institution, typically a husbandry team member, input answers to the corresponding questions and returned the completed survey and weight records to Mystic Aquarium. Questions were designed to assess each of the first three hypotheses proposed.

The individual penguin survey requested that participants sample their population and provide detailed responses for four each (eight total) of normally molting males and females, and all abnormally molting birds. The survey requested animal ID (AZA's Species Survival Program [SSP] maintains identification records of each individual to track lineages for breeding purposes). Other information requested included detailed weight records for 2012-2013, major illnesses (including start date, end date, and diagnosis), and sex. The following specific questions were asked of breeding and nesting: (1) date nests were provided (*i.e.*, when each nesting season began); (2) how many eggs were laid and the date each egg was laid; (3) whether or not the eggs were removed or hatched; and (4) date each egg was removed or hatched. Also, the molt start date (defined as the first observable feather dropped) and end date (defined as last feather dropped) were requested. For abnormally molting penguins, additional questions characterized the nature of the abnormality: (1) failure to molt and year of missed molt; (2) if a partial molt, date first feather dropped, and date feather loss stopped; (3) characterization of feather regrowth-full, partial, beginning and end dates; and (4) written description. Respondents were also asked to describe the nature and start and stop dates of any treatments provided and the response of the bird to said treatment.

The colony survey requested information regarding that institution's colony management practices for 2012 and 2013. It requested the total number of birds in the colony, including the number of abnormally molting birds in each year. Institutions were asked what type of feeding strategy was used, given the options of whether they hand fed, pan fed, or toss fed. Information on vitamin supplementation was requested, which included whether vitamins or supplements were administered, if so, how often (daily or other) and the brand and type of each vitamin or supplement used. Water quality was characterized by asking institutions whether their exhibit contained fresh or salt water. Lighting was considered by asking the institutions if they used fluorescent, halogen, natural, or another type of lighting. Institutions were asked if their penguins were housed in an indoor exhibit, outdoor exhibit, or spent time both indoors and outdoors. To determine the colony's overall molting protocol, the survey questioned if institutions separated molting penguins from the colony, left them in the colony, separated them from the water, separated them from the colony but left with their mate, or if another practice was used. Finally, breeding practices were considered by questioning when and where breeding for the colony took place (exhibit or nesting room; seasonally or yearly). Additionally, the address of each institution was used to place each zoo and aquaria that reported utilizing an indoor and outdoor exhibit in a climate zone based on a climate zone map of the continental United States.

In addition to the survey, the lineage of each individual bird reported was traced back one generation using the African penguin North American regional studbook, dated from 1/1/1950 through 6/24/2015 (compiled by studbook keeper S. Flossic, Tulsa Zoo and Living Museum). All current and previous zoos and aquaria that house(d) each bird were contacted and asked if the bird, identified by studbook number, had ever molted abnormally. An abnormal molt was defined by any one of the following: (1) a molt duration of more than 33 days, (2) incomplete loss or regrowth of feathers; and/or (3) no molt within 1.5 years of the last molt. Criteria one and two define a protracted molt and criterion three defines a missed molt.

Statistical Analyses

Variables were investigated to assess their effects on individuals that experienced normal, protracted, or missed molts. Variables included sex, illness within six months prior to molt, number of eggs laid six months prior to molt, age at molt, pre-molt weight gain or loss (calculated by subtracting the median weight of the year from the closest available recorded weight 2 weeks or less prior to molt start or expected molt start date), and nest to molt interval. A graph of the major life history events for each individual bird was created to provide visual representations for 2012 and 2013.

In order to test the effects of illness within six months prior to molt, number of eggs laid within six months prior to molt, and pre-molt weight gain on the occurrence of abnormal molts, data were selected so as not to artificially inflate sample size from two years of data for each bird. For birds that molted normally in both years, the year to analyze data from was selected randomly for either 2012 or 2013 using the "randbetween" function in Excel. If a bird exhibited a protracted molt both years, or missed a molt both years, the year in which data were used for analysis was also randomly chosen. To ensure sample size was large enough for protracted molt birds, if a bird exhibited a protracted molt one year and a missed molt in another year, the data for the year a protracted molt occurred was used. For birds that only displayed an abnormal molt in one year (either a protracted or missed molt), the data for the year in which the abnormal molt occurred was used. For testing age at molt, the average age between the two years for birds that molted normally in both years was used, whereas the age at which an abnormal molt occurred was used (or the average age of abnormal molt if the bird molted abnormally in the same way [either protracted or no molt] for both years). As above, if a bird exhibited a protracted molt one year and a missed molt in another year, the age for the year a protracted molt occurred was used. In testing the effect of nest to molt interval on abnormal molt, each year was tested independently because the alternative of randomly selecting data by year for a given bird reduced the sample size greatly due to missing data.

Kolmogorov-Smirnov tests were conducted to test for normality. Descriptive statistics, including mean ± standard deviation for normally distributed data, median and interquartile range for non-normally distributed data, and ratios for categorical data were calculated. Nonparametric statistics, including the Kruskal-Wallis test (multiple comparisons with Bonferroni correction at a family $\alpha = 0.2$, Minitab, 2016) and repeated measures ANOVAs on ranked data were used on data sets that were not normally distributed. ANOVA and Tukey's tests were used on data sets that were normally distributed.

Categorical data on the variables of sex and illness six months prior to the expected molt start date were analyzed using a Chi-Squared test. The sex ratio of the normally molting birds of all 17 respondent institutions was used as the expected value for the Chi-Squared test on sex.

Lineage data were tested using a binary logistic regression (BLR) to determine if the prevalence of sires or dams molting abnormally influenced the prevalence of abnormal molting in offspring from the individual survey. All statistical analyses (except the Chi-square tests, which were performed manually), were conducted in Minitab (v. 16.2.4, Minitab Inc., State College, PA), and alpha values (α) were set at 0.05 unless otherwise indicated.

RESULTS

Seventeen zoos and aquaria responded to the survey, reporting data for a total of 176 individuals. In 2012, 14.0% of the 176 birds molted abnormally and in 2013, 13.5% molted abnormally.

Graphs of the major life history events for each individual bird indicated that there may be a difference in the pre-molt weight gain between birds that molted normally and those that molted abnormally (figure 1).

Within the seventeen participating institutions in 2012, the female to male ratio was 0.92:1 for normally molting birds and 0.62:1 for abnormally molting birds. There was not a significant difference in sex between the birds that molted abnormally in comparison to those that molted normally ($x^2 = 1.30$ $df = 1$, $p > 0.1$). In 2013 a female to male ratio of 0.57:1 was observed for normally molting birds and 0.51:1 for abnormally molting birds. There was no difference in sex between abnormally and normally molting birds in 2013 ($x^2 = 0.08$, $df = 1$, $p > 0.5$).

A valid Chi-Square test to evaluate differences in the frequencies of illness within six months prior to the molt between normally and abnormally molting birds could not be conducted due to unacceptably low expected frequencies. For normally molting birds, the ratio of ill to healthy birds was 1:100. Protracted molt birds showed an ill to healthy ratio of 1:17 while birds that did not molt presented a ratio of 1:7.

Institutions reported a median (IQ range) of 0 (0-2) eggs laid within six months prior to molt for normal molters, 0 (0-0) eggs for protracted molters, and 0 (0-1) eggs for non-molters. The number of eggs laid did not differ significantly between the three molt types (Kruskal-Wallis, H = 4.57 [adjusted for ties], $df = 2$, $p = 0.102$).

A reported median (IQ range) for average age at molt showed that normal molters were 11.5 (4.72-21.3) years old, protracted molters were 17.4 (10.3-24.5) years old, and non-molters were 15.1 (7.36-24.5) years old. Birds that did not molt were significantly older than normally molting birds (Kruskal-Wallis, H= 6.15 [adjusted for ties], *df* = 2, $p = 0.046$; figure 2).

Abnormally molting (protracted and no-molt) birds also did not exhibit the same level of pre-molt weight gain seen in normally molting birds (median [IQ range]; normal molt, 0.70 [0.46-0.91] kg; protracted molt, 0.05 [0.00-0.10] kg; no molt, 0.02 [-0.05-0.11]; Kruskal-Wallis, H= 44.20 [adjusted for ties], *df* = 2, $p < 0.001$, figure 3).

In 2012, nest to molt interval differed significantly among normally and abnormally molting birds (mean ± SD; normal molt, 115.6 ± 62.65 d; protracted molt, 19.00 d (n = 1); no molt, 61.00 ± 70.97 d; one-way ANOVA, $F_{2,60} = 3.29$, $p = 0.044$; figure 4), but Tukey's test did not discriminate differences in nest to molt intervals among the three molt types (p > 0.05 in all cases). In 2013 also, nest to molt interval differed significantly among normally and abnormally molting birds (mean ± SD; normal molt, 120.5 ± 76.20 d; protracted molt, 265.5 ± 31.82 d; no molt, 147.4 ± 138.5 d; one way ANOVA, $F_{2,93} = 3.15$, $p = 0.047$, figure 5), but Tukey's test did not discriminate differences in nest to molt intervals among the three molt types ($p > 0.05$ in all cases).

Among abnormally molting birds, 65.6% had an abnormally molting dam as compared to 45.7% of normally molting birds. Also, 53.1% of abnormally molting birds had an abnormally molting sire while 42.9% of normally molting birds did. Binary logistic regression demonstrated that neither the prevalence of sires molting abnormally ($p = 0.382$), dams molting abnormally ($p = 0.858$), nor their interaction ($p = 0.692$) had an effect on the prevalence of their offspring molting abnormally (Table 1).

Vitamin frequency, the use of Mazuri Vita-Zu Small Bird Tablet # 5M25 (PMI Nutrition International, LLC, St. Louis, MO), any Mazuri vitamin type, vitamin E, Vitamin B, water type, natural light, exhibit type, breeding location, and breeding type did not have significant effects on the prevalence of molting abnormalities (Table 2). Climate zone location did have a significant effect on the prevalence of abnormal molt (Table 2). There was also a significant colony variable * year interaction effect for water type and light (Table 2). The effects of feed type (hand, toss, pan), Mazuri Vita-zu bird tablet no vitamin A added # 5TLC, Mazuri Vita-Zu Large Bird Tablet # 5M23, Sea Tabs: Birds, Turtles, Fish, Sharks (Pacific Research Laboratories, Inc, San Diego, CA), fish oil, salt supplement, fluorescent lighting, halogen lighting, LED lighting, outside exhibits, and molting management (left in colony, separated from colony, separated from colony but left with mate) on the prevalence of molting abnormalities in the colonies could not be tested due to small sample sizes within some of the levels of those variables.

DISCUSSION

There was a response rate of 17/54 AZA institutions holding African penguins (31.5%), slightly less than the average response rate for surveys collecting data from organizations (35.7 ± 18.8%, Baruch and Holtom, 2008). Those authors suggest a benchmark response rate within one standard deviation of the average; this study thus meets that benchmark.

Some avian species face time constraints with regard to molting and breeding. Laysan albatrosses (*Diomedea immutablis*) and black-footed albatrosses (*Diomedea nigripes*) only have a four month window available to molt their primary feathers while away from the breeding colony (Langston and Rohwer, 1996). Likewise, for the Emperor penguin (*Aptenodytes forsteri*), the timing of molt and breeding are tightly tuned, and an offset in the timing of one of these events can result in a bioenergetic defecit, affecting the ability to properly prepare for a molt (Groscolas, 1986). The African penguin, however, has a more loosely defined molting and breeding season, molting at any time of the year in Namibia (Kemper *et al*., 2008). This species, then, faces fewer constraints on the timing of both of these energetically expensive life history events.

The life history event of egg laying does not seem to affect the molting process in African penguins. In 2012, normally molting birds seemed to have longer nest to molt intervals than abnormally molting birds, but in 2013, normally molting birds seem to have shorter nest to molt intervals than abnormally molting birds. The results of the effects of nest to molt interval on the prevalence of molting abnormalities are therefore inconclusive. Although the effect of illness experienced within six months prior to the expected molt on the prevalence of molting abnormalities could not be statistically tested, descriptive statistics indicate that normally molting birds seem to have a lower incidence of illness within six months prior to the expected molt than birds that experience protracted or no molts.

While the prevalence of abnormal molters in natural light vs. artificial light exhibits significantly differed from year to year, the results are not consistent between years. Therefore, the effect of exposure to natural light on the prevalence of molt abnormalities is inconclusive. Similarly, although a significant effect of water type * year interaction was seen, there was not a significant main effect of water type. These results are equivocal because the effects may only be present for one of the two years tested.

A higher prevalence of abnormal molts were predicted to occur in climates that were different from the native habitat of the African penguin. However, results demonstrated a lower prevalence of abnormal molting in the humid continental (cool summers) climate zone compared to two others tested (humid subtropical and humid continental, warm summers) that were more similar to the climate zone in the African penguin's native range (Conradie, 2012). However, institutions tested reported using both indoor and outdoor exhibits. It is possible that in the climate zone in which there were colonies of fewer abnormal molters were brought inside more frequently due to the likelihood of more winter extremes existing in this climate zone.

This study also indicates that an abnormal molt is not a heritable trait. Research suggests a genetic component to molt in other avian species such as African stonechats (*Saxicola torquata axillaris,* Helm and Gwinner, 1999). Among Stonechat subspecies, a genetic selection in response to environmental conditions is exhibited. However, this study suggests otherwise in the African penguin.

Pre-molt weight gain appears to be a significant factor affecting abnormal molt, both protracted and absent. According to Cooper (1978), pre-molt African penguins gain 31% of their body mass in preparation for the molt. However, abnormally molting birds in the present study only gained on average approximately 8% (2012) and 7% (2013) of their average weight prior to the start or expected start of molt. Additionally, although birds housed in zoos and aquaria often times still have access to food throughout the molt, they tend to lose appetite and may refuse food completely during the molt (Mazzaro *et al*., 2013). In other penguin species, such as the adult Emperor and King penguins (*Aptenodytes forsteri* and *A. patagonicus*, respectively), a two-fold increase in energy expenditure and a two-fold decrease in body mass during molt has been recorded (Groscolas and Cherel, 1992). Therefore, if a bird is unable to gain the essential weight to support such an energetically expensive process, it could result in an abnormal progression or the process not occurring at all.

Birds that missed molts were also significantly older than birds with normal molts. Abnormal molting may thus be a disorder associated with aging, such as Alzheimer's disease or osteoporosis in humans. The mean life expectancies for African penguins in the wild are 17.3 and 15.1 years for males and females, respectively (Mystic Aquarium). The life expectancy for birds housed in zoos and aquaria can reach 25 years (Mystic Aquarium, unpublished data). Therefore, the average age of birds that molted abnormally can be considered near the end of the average life expectancy for the species. As birds age, their bodies may be unable to secrete hormones and maintain concentrations at effective levels. Because of the significant difference in weight gain between normal and abnormal birds, the thyroid in particular may be playing a key role in abnormal molt. The thyroid is known to regulate metabolism, has an intricate relationship with body mass, and can affect the progression of molt in the avian species. Thyroidectomy of Turkey hens at various life stages prevented molting. However, providing the hens with 1ppm of T_4 induced molting, indicating that thyroid is an essential factor for molting in hens (Lien and Siopes, 1989). Other studies of penguin species indicate that thyroid hormone thyroxine (T_4) has a significant impact on molt regulation. During the pre-molt stage, T_4 rises and stimulates new feather growth (Groscolas and Cherel, 1992; Otsuka *et al*., 2004).

In human subjects, functional changes such as decreased T_3 and thyroid-stimulating hormone (TSH) concentrations, have been observed with aging (Chiovato *et al*., 1997; Mariotti *et al*., 1995). In the African penguin, an inability to maintain effective thyroid hormone concentrations as the bird ages may affect the ability to gain weight prior to the molt, and thus the ability to synthesize new feathers, all leading to the likelihood of an abnormal molt.

Recommendations

These results yield some sensible recommendations to veterinarian and husbandry teams to focus on preventative care, treatment, and husbandry practices aimed at supporting a healthy molt in African penguins. With no relationships observed between colony management strategies and the occurrence of abnormal molts, needed modifications of habitats are not indicated. Similarly, because it is indicated that abnormal molt is not hereditary, selective breeding to avoid molting abnormalities in offspring is not indicated.

Based on results, husbandry and veterinary care teams are advised to closely monitor weight through consistent measurements. After the first juvenile molt, an expected molt start date can be approximated. Care teams should monitor weight prior to the expected start date and determine if the bird is gaining an appropriate prevalence of its body weight. If the bird is not doing so, the husbandry and veterinary team may consider supplementing the bird's diet to ensure proper weight gain in order to support a healthy molt.

Furthermore, because birds that molted abnormally were significantly older than those that molted normally, zoos and aquaria are advised to closely monitor aging birds in particular, especially near the expected start date of the annual molt. Dependent upon the bird's condition, supplements or other medical treatments could be used to support a healthy and complete molt.

In aquarium-maintained Chinstrap penguins (*Pygoscelis antarctica),* six non-molting birds were given two series of injections of medroxyprogesterone acetate, a synthetic variant of the steroid hormone progesterone, which successfully induced molt in all birds (Reidarson *et al*., 1999). Induction of catastrophic molt was also successfully accomplished in four Yellow-eyed penguins (*Megadyptes antipodes*) with the oral supplementation of fresh beef thyroid (Aguilar, 2015). Further research should be conducted to determine 1) if thyroid function decreases with aging in African penguins, 2) if insufficiently maintained concentrations of T_3 and T_4 prior to and during molt is the cause behind a lack of pre-molt weight gain, and therefore, abnormal molts, and 3) safe and effective protocols for hormone replacement therapy. Determination of the effects that caloric intake and hormone secretion and maintenance have on abnormal molt will further enable veterinary and husbandry teams to provide preventive care and treatment for abnormally molting birds. Although a more challenging scenario in the wild, wildlife management teams could use this information to help support the wild African penguin population. SANCCOB and other rehabilitation groups who encounter abnormally molting birds could provide them with nutritional support and the hormone replacement treatment that supports a healthy molt progression.

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Predictor	b	se	z ratio	p	odds
Sire	0.606	0.693	0.88	0.382	1.83
Dam	0.136	0.763	0.18	0.858	1.15
Sire*Dam	0.403	1.02	0.4	0.692	1.5
Constant	0	0.508	-1.19	0.232	

Table 1. Summary of binary logistic regression analysis of the effect of the prevalence of abnormal molt by the sire, dam, or their interaction on the prevalence of abnormal molting in their offspring. Logistic regression coefficients and standard error are abbreviated by “b” and “se” respectively.

		Colony Variable			Colony Variable*Year Interaction		
Variable	Percentage of abnormal molters median (IQ Range, %)	df (num, denom)	F	p	df (num, denom)	F	p
Vitamin Frequency		1,15	0.03	0.857	1,15	0.29	0.596
Daily	12.0 (7.45-19.6)						
Other	14.0 (10.7-19.7)						
Mazuri Vita-Zu Small Bird Tablet # 5M25		1,15	1.44	0.249	1,15	1.19	0.292
Yes	17.0 (12.2-24.7)						
No	10.7 (8.70-17.7)						
Any Mazuri Vitamin Type		1,15	0.67	0.425	1,15	0.19	0.666
Yes	15.8 (10.7-20.2)						
No	8.73 (7.13-16.6)						
Vitamin E		1,15	0.01	0.912	1,15	0.43	0.521
Yes	12.1 (8.70-32.8)						
No	13.7 (10.2-19.5)						
Vitamin B		1,15	0.40	0.539	1,15	1.21	0.290
Yes	15.9 (10.5-37.5)						
No	12.02 (8.71-19.1)						
Water Type		1,15	0.00	0.953	1,15	6.48	**0.022**
2012							
Fresh	11.5 (7.55-16.7)						
Salt	15.8 (11.0-29.6)						
2013							
Fresh	17.4 (8.77-20.7)						
Salt	12.8 (8.04-15.9)						

Natural Light		1,15	0.00	0.945	1,15	13.63	**0.002**
2012							
Yes	11.1 (8.31-16.7)						
No	20.5 (11.1-22.2)						
2013							
Yes	18.1 (10.2-22.3)						
No	11.1 (8.77-14.9)						
Exhibit Type		2,14	0.4	0.677	2,14	1.98	0.175
Inside	16.8 (10.7-34.9)						
Both inside and outside	11.3 (7.83-17.21)						
Breeding Location		1,15	0.02	0.889	1,15	1.98	0.18
Exhibit	14.0 (10.7-18.8)						
Nesting room	11.3 (8.41-21.07)						
Breeding Type		1,15	4.41	0.053	1,15	2.17	0.161
Seasonal	19.8 (15.3-25.6)						
Yearly	10.91 (7.45-17.73)						
Climate Zone		2,7	12.11	**0.005**	2,7	0.47	0.644

Table 2. The effects of colony management variables on the prevalence of abnormally molting penguins at participating institutions. Descriptive statistics (median [IQ range]) and results of repeated measures ANOVAs on ranked data are reported (results of the main factor of year are excluded). Significant effects ($p < 0.05$) in bold.

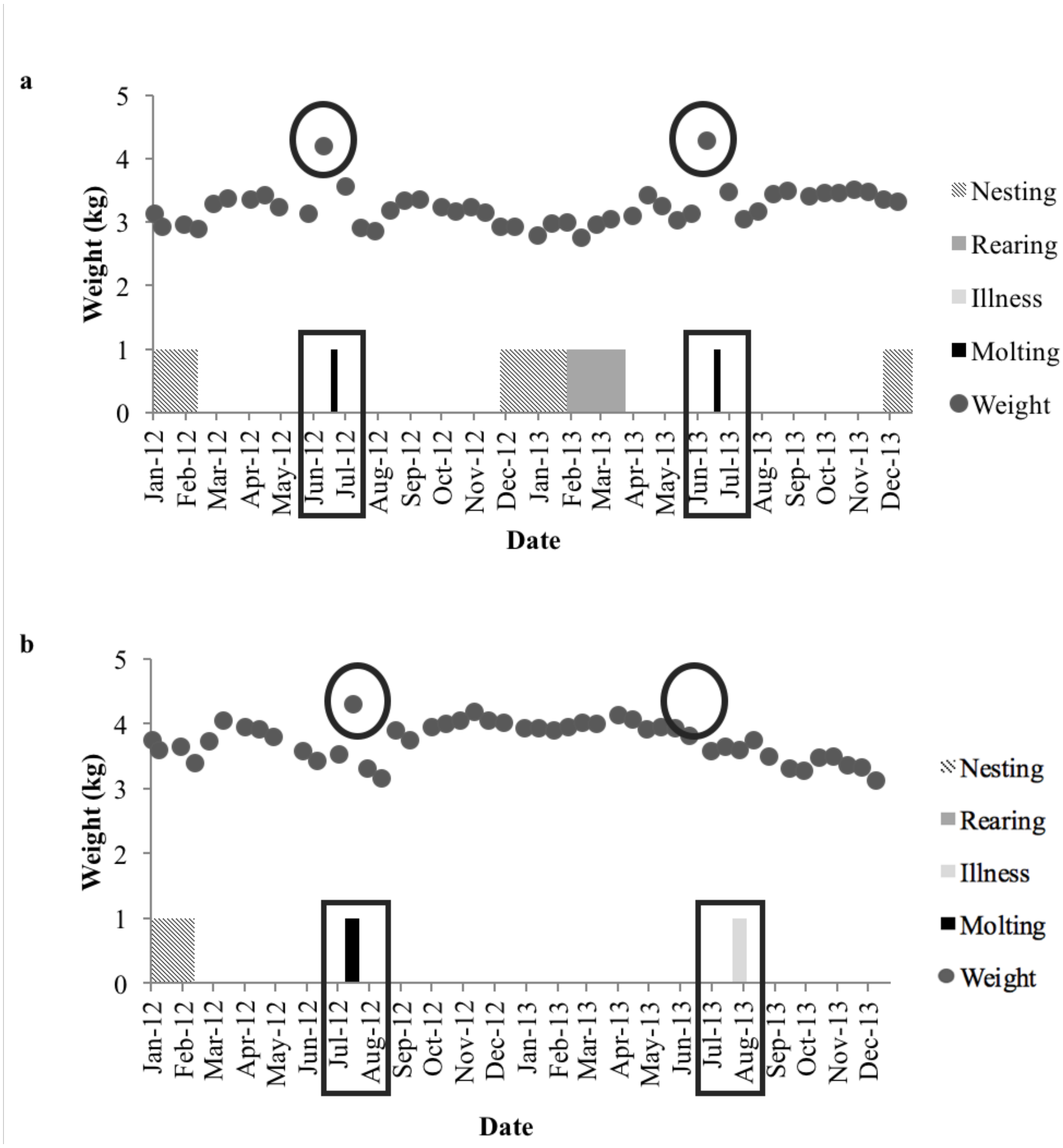

Figure 1. A visual representation of the major life history events in 2012 and 2013 for a) a normally molting and b) abnormally molting male African penguin. Boxes and circles indicate how the normally molting bird experienced an annual molt accompanied by a pre-molt weight gain whereas the abnormal bird did not molt in 2013 (expected molt start date estimated from normal 2012 molt date) or a experience a weight gain.

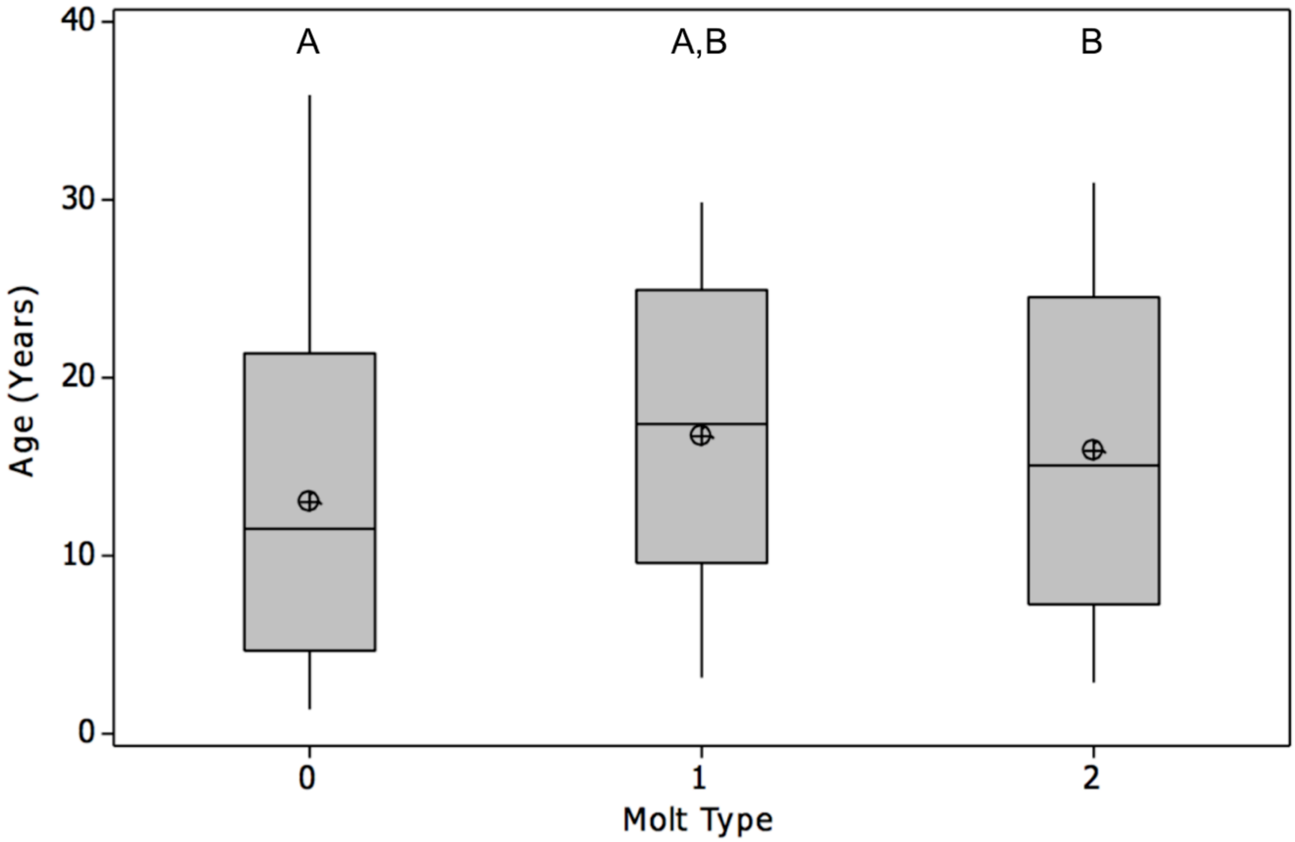

Figure 2. Box plot of age at molt in 2012 or 2013 for normally molting (0), protracted molting (1), and non-molting (2) birds. Box plots that do not share the same letter are significantly different (multiple comparison test with Bonferroni correction, $p < 0.067$).

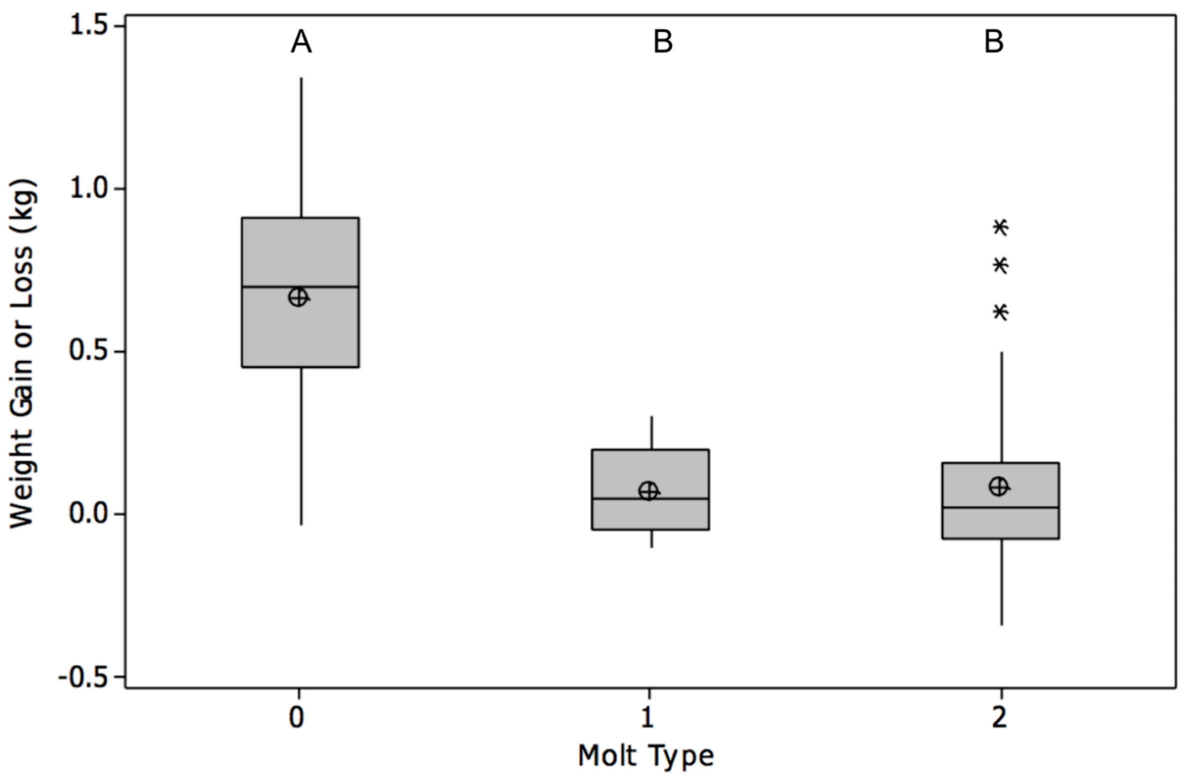

Figure 3. Box plot of weight gain or loss in 2012 or 2013 for normally molting (0), protracted molting (1), or non-molting (2) birds. Box plots that do not share the same letter are significantly different (multiple comparison test with Bonferroni correction, $p < 0.067$). * represents outliers.

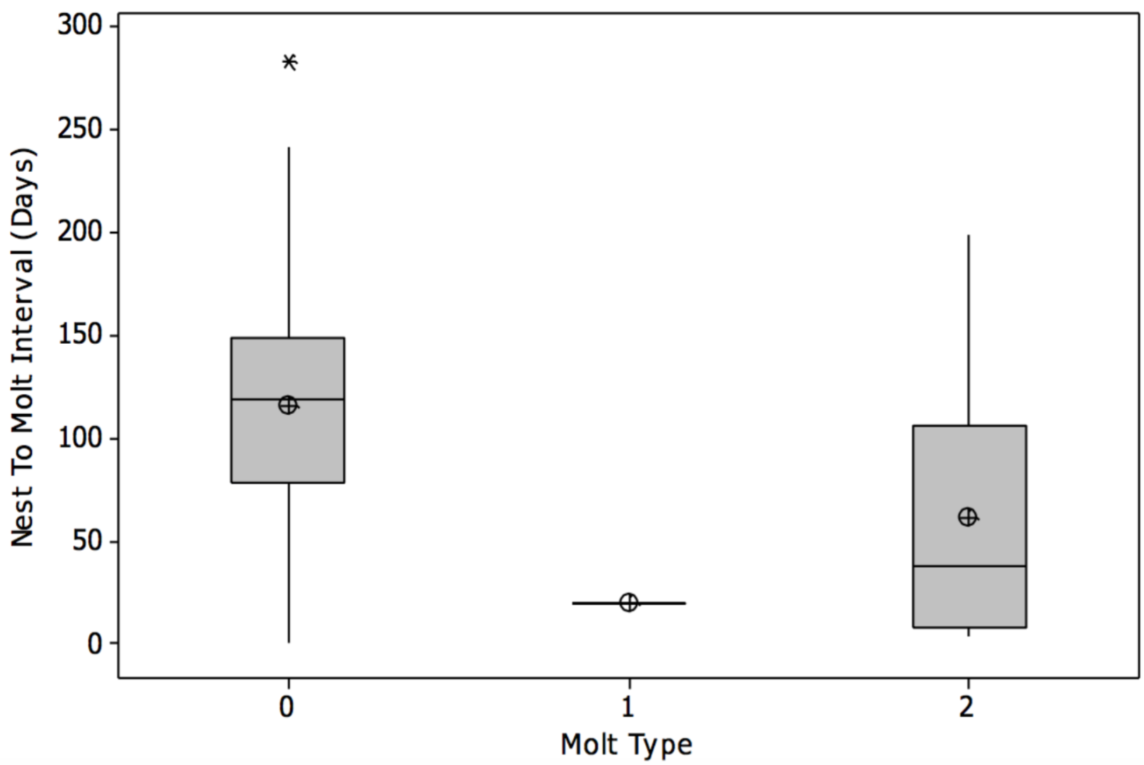

Figure 4. Box plot of nest to molt interval in normally molting (0), protracted molting (1), and non-molting (2) birds in 2012. * represents outliers.

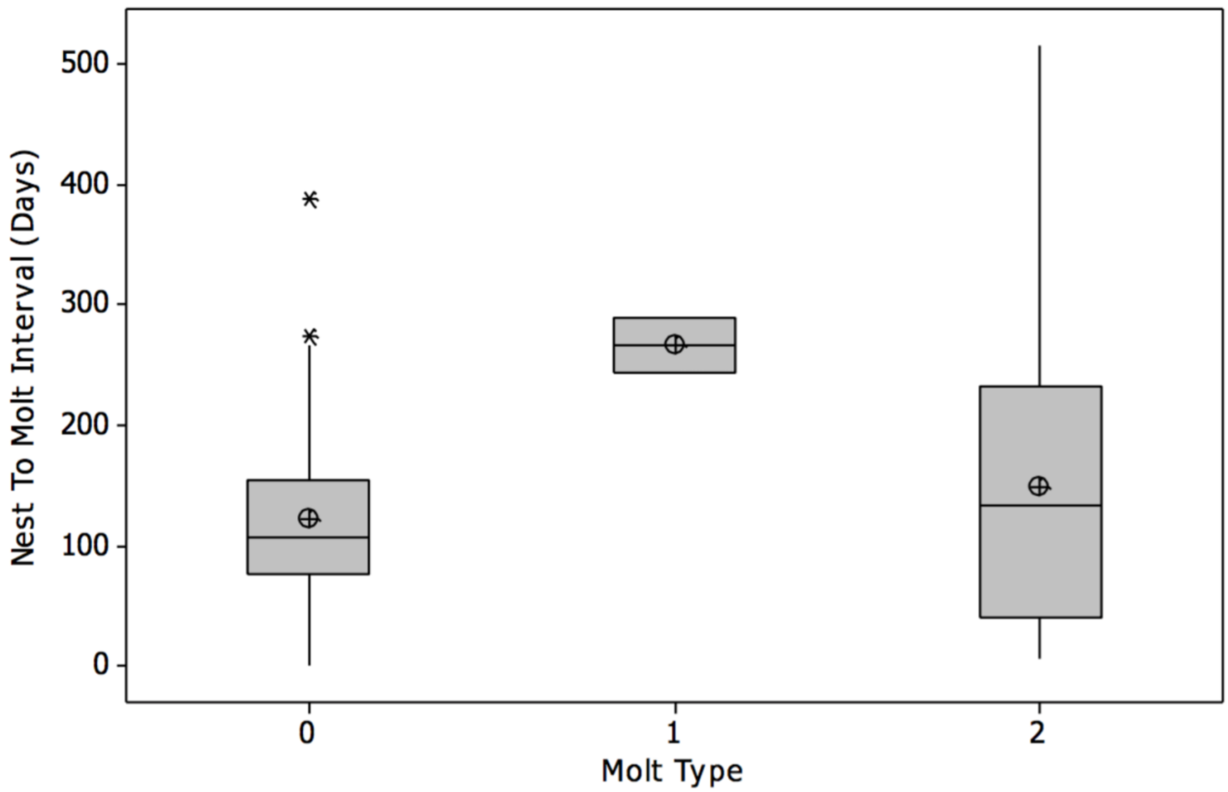

Figure 5. Box plot of nest to molt interval in normally molting (0), protracted molting (1), and non-molting (2) birds in 2013. * represents outliers.